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Human Genome Epidemiology (HuGE) Review

Pooled Analysis and Meta-analysis of the *Glutathione* S-*Transferase P1 Ile 105Val* Polymorphism and Bladder Cancer: A HuGE-GSEC Review

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The *glutathione* S-*transferase P1* genotype (*GSTP1*) is involved in the inactivation of cigarette smoke carcinogens, and sequence variation in the gene may alter bladder cancer susceptibility. To examine the association between *GSTP1Ile 105Val* and bladder cancer, the authors undertook a meta- and pooled analysis. Summary crude and adjusted odds ratios and corresponding 95% confidence intervals were pooled by using a random-effects model. In the meta-analysis (16 studies, 4,273 cases and 5,081 controls), the unadjusted summary odds ratios for *GSTP1 Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile* were 1.54 (95% confidence interval: 1.21, 1.99; $p < 0.001$) and 2.17 (95% confidence interval: 1.27, 3.71; $p = 0.005$). The association appeared to be the strongest in Asian countries. When the analysis was limited to European descendents (nine studies), the summary odds ratio decreased (odds ratio = 1.24, 95% confidence interval: 1.00, 1.52) ($Q = 17.50$; $p = 0.02$). All relevant data previously contributed to the International Study on Genetic Susceptibility to Environmental Carcinogens were pooled (eight studies, 1,305 cases and 1,558 controls). The summary odds ratios were similar to the ones from the meta-analysis. Case-only analyses did not detect an interaction between the *GSTP1* genotype and smoking status (never/ever). *GSTP1 Ile 105Val* appears to be associated with a modest increase in the risk of bladder cancer.

epidemiology; GSTP1; meta-analysis; urinary bladder neoplasms

Abbreviations: CI, confidence interval; GSEC, Genetic Susceptibility to Environmental Carcinogens; *GSTP1*, *glutathione* S-*transferase P1* genotype; OR, odds ratio.

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Editor's note: *This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).*

GENE AND GENE VARIANTS

The glutathione *S*-transferases comprise a supergene family of phase II detoxifying enzymes that catalyze a variety of reduced glutathione-dependent reactions with compounds containing an electrophilic center (1). The glutathione *S*-transferase family is involved in the metabolism of a wide range of chemicals including environmental carcinogens, reactive oxygen species, and chemotherapeutic agents. Glutathione *S*-transferase provides protection because individual glutathione *S*-transferase genes are each regulated in a distinct fashion and each encodes a protein with unique catalytic activity (2). In humans, eight distinct gene families have been identified: α on chromosome 6, μ on chromosome 1, θ on chromosome 22, π on chromosome 11, ζ on chromosome 14, σ on chromosome 4, κ (chromosomal location not known), and χ (also called ω) on chromosome 10 (3).

Glutathione S-*transferase P1* (*GSTP1*), belonging to the π class gene family, is involved in the inactivation of cigarette smoke carcinogens, such as benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) and other diol epoxides of polycyclic aromatic hydrocarbons (4). Two single nucleotide polymorphisms have been described. The first is an *A*-to-*G* substitution at base pair 313 at codon 105 resulting in an amino acid difference, from isoleucine to valine (5). It has been shown that the activity of the isoleucine 105 variant toward several carcinogenic diol epoxides is lower compared with that of the valine 105 form (6, 7). This result was recently confirmed by the finding that *GSTP1 Val* possesses up to fivefold more enzymatic activity to some polycyclic aromatic hydrocarbons in *GSTP1 Ile/Val* or *Ile/Ile* (8). The second polymorphism is a nucleotide substitution of *C* to *T* that results in alanine to valine at codon 114. So far, three functional variants have been identified: *GSTP1*A* (*105 Ile*;*114Ala*), *GSTP1*B* (*105Val*; *114Ala*), and *GSTP1*C* (*105Val*;*114Val*) (9).

GENE VARIANT FREQUENCY

A meta-analysis of the association between *GSTP1* and the risk of prostate cancer found that the frequency of the *GSTP1 105Val* allele in controls was 32 percent (95 percent confidence interval (CI): 31, 33) for those of European descent and 14 percent (95 percent CI: 9, 19) for those of Asian descent (10). Overall, the prevalence of *Val/Val* homozygosity was 11 percent and 0 percent in controls of European and Asian descent, respectively. The respective prevalence rates of *Ile/Val* heterozygosity were 43 percent and 28 percent (10). A meta-analysis that described the relation between *GSTP1* and the risk of acute leukemia found that the frequency of the *GSTP1 105Val* genotype in the controls was 65.2–75.4 percent in Europeans and 65.2–68.4 percent in persons in the United States, Canada, and Brazil (11). A meta- and pooled analysis of *GSTP1* and the risk of head and neck cancer mentioned that the frequency of the *GSTP1 Val* genotype among controls was 23.8–64.6 percent (12).

DISEASE

An estimated 357,000 cases of bladder cancer occurred worldwide in 2002, making this the ninth most common cause of cancer for both sexes combined. There were 145,000 deaths, with population-based 5-year survival rates ranging from 40 percent to 80 percent depending on whether noninvasive lesions are included in the computation. Bladder cancer is relatively common in high-income countries, where 63 percent of all incident cases are diagnosed. The majority (77 percent) of bladder tumors occur in men. International incidence rates vary. High incidences are found in many European countries and in parts of Africa and the Middle East, where bladder cancer is associated with chronic *Schistosoma hematobium* infection (13). Bladder cancer is a heterogeneous disease with a variable natural history. Low-grade tumors have a low progression rate and require initial endoscopic treatment and surveillance, but they rarely present a threat to the patient. Alternatively, high-grade tumors have a high malignancy potential associated with significant progression and cancer death rates (14).

Tobacco is the main risk factor. Approximately half of the cases of male urinary tract cancer and one third of the cases of female urinary tract cancer might be attributable to cigarette smoking (15). Occupational exposure, particularly to aromatic amines and polycyclic aromatic hydrocarbons, may play an important role in perhaps 10 percent of bladder cancers (16). Consumption of fresh fruits and vegetables may provide important protection (16).

Several genetic susceptibility factors have been studied in relation to bladder cancer. A previous Human Genome Epidemiology (HuGE) review concluded that *GSTM1* null status, involved in the detoxification of polycyclic aromatic hydrocarbons, is associated with a modest increase in the risk of bladder cancer, with a summary odds ratio of 1.44 (95 percent CI: 1.23, 1.68) (17). *N*-acetyltransferase 2 is involved in the biotransformation of aromatic and heterocyclic amines. It has been suggested that slow acetylation is associated with a small increase in bladder cancer risk (odds ratio (OR) = 1.31, 95 percent CI: 1.11, 1.55) (18).

A meta-analysis of 16 studies published up to 1999 observed an interaction between smoking and *N*-acetyltransferase 2 slow acetylation (OR = 1.3, 95 percent CI: 1.0, 1.6) (19). Furthermore, an increased percentage of *N*-acetyltransferase 2 slow acetylators has been reported for cases of bladder cancer in European descendents occupationally exposed to aromatic amines (20).

A recently updated meta-analysis of *N*-acetyltransferase 2 and bladder cancer, including 5,091 cases and 6,501 controls, found that the summary relative risk for *N*-acetyltransferase 2 slow acetylators compared with rapid/intermediate acetylators was 1.4 (95 percent CI: 1.2, 1.6). An updated meta-analysis of *GSTM1* found that the summary odds ratio for *GSTM1* null versus present phenotype was 1.5 (95 percent CI: 1.3, 1.6) (21). Mechanistic studies have demonstrated that

the urinary bladder epithelium is well capable of metabolic activation reactions with respect to both aromatic amines and polycyclic aromatic hydrocarbons. This finding is consistent with a *GSTM1* interaction with smoking regarding urothelial carcinogenesis if polycyclic aromatic hydrocarbons are involved (22). (The effect of the *N*-acetyltransferase 2 polymorphism on urinary bladder cancer seems to differ between monoarylamines and aryldiamines (23).)

ASSOCIATIONS AND INTERACTIONS

Because of the detoxification role of polycyclic aromatic hydrocarbons present in tobacco and certain occupational exposures, *GSTP1* enzymes and the genes encoding them may play an important role in modifying bladder cancer susceptibility. Most of the studies conducted have been rather small with limited statistical power, and potential interaction with smoking has not been properly investigated. We undertook a meta- and pooled analyses of all identified studies to quantify the association between *GSTP1 Ile 105Val* and bladder cancer to determine potential sources of heterogeneity among the study results and to explore gene-environment interactions.

META-ANALYSIS

Search strategy

A computerized search of Medline (National Library of Medicine, Bethesda, Maryland) (1966–2006), Embase (Elsevier B. V., Amderstam, the Netherlands (1974–2006), and Current Contents (Thomson Scientific, Philadelphia, Pennsylvania) (1998–2006) was conducted by two independent researchers (E. K., M. H.) to identify published epidemiologic studies related to bladder cancer and *GSTP1 Ile 105Val*. The medical subject headings (MeSH; National Library of Medicine, Bethesda, Maryland) ''bladder neoplasm,'' ''urologic neoplasm,'' ''genetic polymorphism,'' ''glutathione *S*-transferase,'' and the free-text words ''GST,'' ''GSTP1'' were combined. No language or other restrictions were placed on the search. Furthermore, references cited in published original and review articles (2, 4, 24) were examined until no further study was identified. Authors of retrieved articles were contacted where necessary and were asked to provide additional information. To reduce the risk of publication bias, all International Bladder Cancer Consortium participants were invited to identify eligible published or unpublished studies.

Inclusion/exclusion criteria

Articles from peer-reviewed medical journals were included if they reported on studies using a case-control, cohort, nested case-control, or cross-sectional design and provided sufficient data to calculate an odds ratio and corresponding 95 percent confidence interval.

Data extraction

E. K. and M. H. independently reviewed all studies and abstracted data by using a standardized form. For all papers, one researcher (M. H.) was blinded to the author(s), title of the journal, year of publication, references, acknowledgments, and associations.

Qualitative data extraction

Study characteristics extracted from each paper included country, year of publication, design (cohort, nested case-control, case-control), ethnicity (defined as of European descent, Black, Asian, Hispanic, or unknown), setting (population, hospital), number of cases and controls/cohort, mean ages of cases and controls/cohort, rate of each sex for cases and controls/cohort, degree of participation for cases and controls/cohort, and stage and invasiveness of disease. Any disagreement between researchers was resolved by continuing discussions until a consensus was reached (original disagreement was <1 percent for all data extracted).

Quantitative data extraction

The odds ratios describing the relation between *GSTP1 Ile 105Val* status and risk of bladder cancer were the major outcomes of the study. Data were extracted to permit calculation of a crude odds ratio. Two-way contingency tables for each study were constructed, based on exposure frequency distributions, to calculate the unadjusted odds ratio. Adjusted odds ratios were extracted directly from the original reports.

Statistical analysis

Summary crude and adjusted odds ratios and corresponding 95 percent confidence intervals were pooled by using a random-effects model. The random-effects approach allows for heterogeneity in studies beyond sampling error by adding an empirical estimate of the between-study variance to the within-study variance. The Q statistic, which measures homogeneity between studies, was used to determine the presence of heterogeneity (25). Possible sources of heterogeneity were explored by using meta-regression analysis to examine the influence of the following study characteristics: region (Europe, United States, and Asia), ethnicity, publication year, and method of selecting the controls (hospital vs. population). Meta-regression extends a random-effects meta-analysis to estimate the extent to which one or more study-level covariates explain heterogeneity. Meta-regression first models by using two additive components of variance: one representing the variance within units and the other the variance between units.

In outlier analysis, we examined the influence of every study on the summary odds ratio by repeatedly pooling the odds ratio while excluding one study each time. Studies contributing the most to the heterogeneity were removed sequentially until homogeneity was achieved. Publication bias was investigated both visually by using a funnel plot and statistically via Egger's unweighted regression test, which measures the degree of funnel plot asymmetry (26). All analyses were performed by using STATA statistical software, version 8.0 (27).

POOLED ANALYSIS

We included all relevant data available from the database maintained by the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC) (28, 29). This database contains individual-level data from case-control studies on genes that metabolize environmental carcinogens. Our data included the original data from 10 studies, eight case-control studies of which are also included in the meta-analysis (30–37), and one unpublished case-control study (K. Golka, unpublished study). These data comprised 1,305 cases and 1,558 controls. For some studies, the numbers of cases and controls did not completely match those reported in the publications (32, 33, 37). One data set (36) did not provide the results of genotyping for *GSTP1 Ile 105Val* for the controls and was therefore included in the case-only analyses.

In statistical analyses, we estimated study-specific odds ratios and their 95 percent confidence intervals to assess the association between the *GSTP1 Ile 105Val* polymorphisms and bladder cancer risk. Crude odds ratios and odds ratios adjusted for sex, age, and smoking status (never/former/current) were calculated by logistic regression models. To estimate whether the differences in study-specific odds ratios were greater than could be expected by chance, a Q test for heterogeneity was performed. A summary odds ratio was estimated by the random-effects model, since heterogeneity was present among the studies. Because the data could be affected by inclusion bias, Egger's test and funnel plots were performed. Case-only analyses were conducted to examine a multiplicative interaction between smoking (never/former/current) and the different *GSTP1 Ile 105Val* polymorphisms. If it is assumed that the environmental exposure and genetic factors occur independently, analyses of case-only studies are more precise for estimating gene-environment interactions than those based on cases and controls (smaller standard errors due to elimination of control group variability) (38). A case-only odds ratio greater than 1 would indicate that the relation between smoking and bladder cancer is stronger among *GSTP1 Ile/Val* and *Val/Val* subjects than among *GSTP1 Ile/Ile* subjects. Conditional logistic regression was used to calculate the odds ratio for *GSTP1 Ile 105Val* genotype stratified by *GSTM1* and *GSTT1* status.

RESULTS

Meta-analysis

Literature search and study characteristics. The search strategy identified 23 epidemiologic studies reporting on the association between *GSTP1 Ile 105Val* and bladder cancer (21, 30–37, 39–52). Seven studies were excluded because *GSTP1* was determined as a tumor marker (46–52). The remaining 16 articles described 15 case-control studies (21, 30, 31, 33–37, 39–45) and one nested case-control study (32), comprising 4,273 cases and 5,081 controls. Population-based controls were assessed in seven studies (30, 33–35, 39–41). Seven studies were carried out in European countries (21, 30, 31, 36, 37, 41, 44), three in American countries (39, 40, 45), five in Asian countries (32–34, 42, 43), and one in north Africa (35). In all of the studies, *GSTP1* status was determined by polymerase chain reaction assays. The distribution of *GSTP1 105Val* was consistent with Hardy-Weinberg equilibrium in eight studies (21, 30, 31, 34, 36, 42–44).

Eight studies did not mention the Hardy-Weinberg equilibrium (32, 33, 35, 37, 39–41, 45). All studies were published in English. Ethnicity was detailed in 13 of the studies (21, 30–33, 35–37, 39–41, 44, 45). Most of the subjects were either European descendents (56.25 percent) or Asian (12.50 percent).

The frequency of *Val/Val* homozygosity was 14 percent in controls of European descendents and 5 percent in those of Asian origin. The respective frequency rates of *Ile/Val* heterozygosity were 40 percent and 32 percent in the controls.

Effect-size estimation. The results of the meta-analysis are presented in table 2 and in figures 1 and 2.

The unadjusted summary odds ratio for *GSTP1 Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile* was 1.44 (95 percent CI: 1.17, 1.77; $p < 0.001$). Heterogeneity was statistically significant between these studies ($Q = 46.21$; $p < 0.001$). Meta-regression analyses examined the influences of five covariates: publication year ($p = 0.58$), ethnicity ($p = 0.11$), region ($p = 0.15$), study design ($p = 0.54$), and method of selecting controls ($p = 0.46$). Heterogeneity was no longer significant after summary unadjusted odds ratios were calculated for the three different regions (Europe, United States, and Asia). Egger's unweighted regression test suggested publication bias ($p = 0.003$).

Restriction to studies with at least 100 cases and 100 controls (11 studies) conferred a summary odds ratio of 1.42 (95 percent CI: 1.11, 1.82; $p = 0.006$) ($Q = 39.30$; $p < 0.001$). Restriction to studies that used population controls (seven studies) did not alter the summary odds ratio substantially (OR = 1.46, 95 percent CI: 1.02, 2.08; $p = 0.04$) ($Q = 26.09$; $p < 0.001$). By limiting the analysis to European descendents (nine studies), the summary odds ratio decreased (OR = 1.24, 95 percent CI: 1.00, 1.52; $p = 0.04$) ($Q = 17.50$; $p = 0.02$) (data not shown).

We performed an influence analysis, in which the meta-analysis estimates are computed by omitting one study in each turn, to investigate the influence of a single study on the overall estimate. Two potentially outlying studies were identified (34, 43). After these studies were excluded, the unadjusted summary odds ratio for *GSTP1 Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile* was 1.25 (95 percent CI: 1.06, 1.48; $p = 0.005$) ($Q = 23.15$; $p = 0.009$).

The unadjusted summary odds ratios for *GSTP1 Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile* were 1.54 (95 percent CI: 1.21, 1.99; $p < 0.001$) for 13 studies and 2.17 (95 percent CI: 1.27, 3.71; $p = 0.005$) for 12 studies.

The adjusted summary odds ratio for *GSTP1 Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile* was 1.67 (95 percent CI: 1.14, 2.44; $p = 0.008$). Heterogeneity was not statistically significant between these three studies ($Q = 0.27$; $p = 0.87$). No outlying studies were identified through an influence analysis. The adjusted summary odds ratio for *GSTP1 Ilel/Val* compared with *GSTP1 Ile/Ile* was 1.45 (95 percent CI: 0.92, 2.29; $p = 0.11$) ($Q = 26.95$; $p < 0.001$) for seven studies. Meta-regression analyses examining the influences of four covariates found that ethnicity had a significant

TABLE 1. Characteristics of the studies used in the pooled and meta-analysis of *GSTP1 and bladder cancer risk**

Author(s), year of publication (reference no.)	Country	Study design	No. of cases	No. of controls	Source of controls	Unadjusted OR* for *AG*/*GG* vs. *AA*	95% CI*
Ma et al., 2003 (32)	China	Nested case-control	23	210	Former benzidine-exposed workers	2.00	0.76, 5.27
Ma et al., 2002 (33)	China	Case-control	61	182	Population	1.94	0.99, 3.8
Broberg et al., 2005 (30)	Sweden	Case-control	63	158	Population	1.30	0.68, 2.50
Harries et al., 1997 (41)†	United Kingdom	Case-control	71	155	Population	1.91	1.03, 3.58
Saad et al., 2005 (35)	Egypt	Case-control	72	82	Population	0.93	0.47, 1.84
Katoh et al., 1999 (42)†	Japan	Case-control	106	122	Hospital	1.37	0.72, 2.60
Mittal et al., 2005 (34)	India	Case-control	106	162	Population	3.14	1.81, 5.45
Srivastava et al., 2005 (43)†	India	Case-control	106	370	Unknown	2.36	1.46, 3.86
Toruner et al., 2001 (44)†	Turkey	Case-control	121	121	Hospital	1.76	1.01, 3.08
Steinhoff et al., 2000 (36)	Germany	Case-control	135	127	Hospital	1.25	0.75, 2.09
Cao et al., 2005 (45)†	United States	Case-control	145	170	Blood donors	4.95	1.05, 46.48
Gago-Dominguez et al., 2003 (40)†	United States	Case-control	159	163	Population	1.06	0.66, 1.69
Peluso et al., 2000 (37)	Italy	Case-control	162	104	Hospital	1.65	0.97, 2.81
Hung et al., 2004 (31)	Italy	Case-control	201	214	Hospital	1.04	0.70, 1.56
Unpublished study of Golka‡	Germany	Case-control	216	201	Hospital		
Garcia-Closas et al., 2005 (21)†	Spain	Case-control	1,150	1,149	Hospital	1.01	0.85, 1.20
Castelao et al., 2004 (39)†	United States	Case-control	1,592	1,592	Population	0.87	0.67, 1.14

* *GSTP1*, *gluthatione* S-*transferase P1* genotype; OR, odds ratio; CI, confidence interval.
† Included in the meta-analysis only.
‡ Included in the pooled analysis only.

TABLE 2. Meta-analysis summary odds ratios for *GSTP1 *Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile***

	Unadjusted summary OR*					
	No. of studies analyzed	OR	95% CI*	*p* value	*Q*†	*p* value†
GSTP1 Ile/Val and *Val/Val* vs. *Ile/Ile*						
All regions	16	1.44	1.17, 1.77	<0.001	46.21	<0.001
Europe	7	1.23	1.04, 1.57	0.02	10.25	0.11
United States	3	1.09	0.68, 1.77	0.71	5.17	0.07
Asia	6	1.87	1.31, 2.66	<0.001	10.52	0.06
GSTP1 Ile/Val vs. *Ile/Ile*						
All regions	13	1.54	1.21, 1.99	<0.001	32.80	<0.001
Europe	6	1.27	1.00, 1.59	0.04	7.78	0.17
United States	1					
Asia	6	1.73	1.17, 2.55	0.006	11.63	0.04
GSTP1 Val/Val vs. *Ile/Ile*						
All regions	12	2.17	1.27, 3.71	0.005	41.53	<0.001
Europe	6	1.58	0.88, 2.85	0.13	13.77	0.02
United States	1					
Asia	5	2.97	1.26, 7.03	0.01	11.03	0.03

* *GSTP1*, *gluthatione* S-*transferase P1* genotype; OR, odds ratio; CI, confidence interval.
† χ^2 test for heterogeneity.

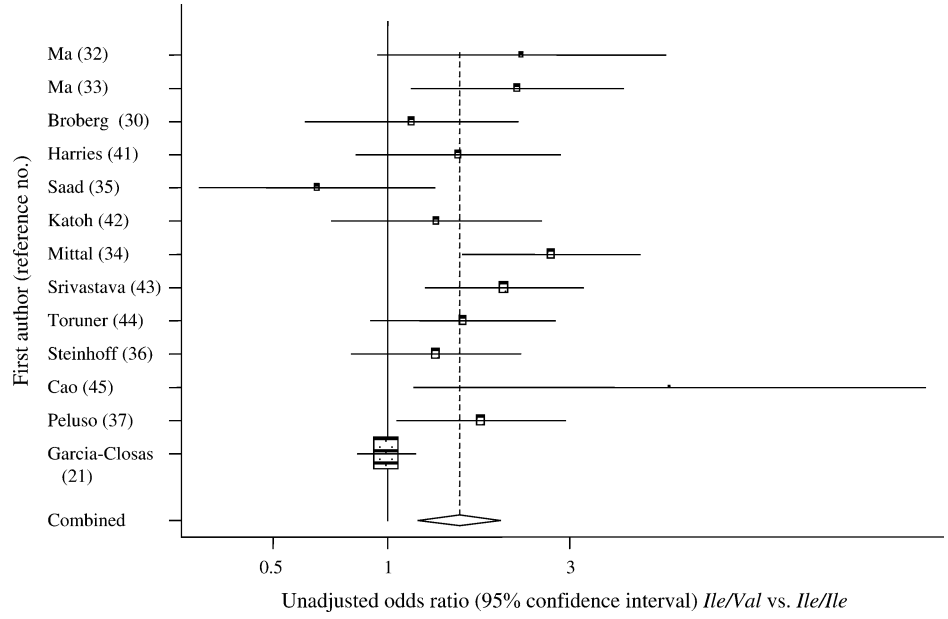

FIGURE 1. Forest plot of unadjusted odds ratios and corresponding 95% confidence intervals for *glutathione* S-*transferase P1 Ile 105Val* (*GSTP1 Ile/Val*) versus *glutathione* S-*transferase P1 Ile 105Ile* (*GSTP1 Ile/Ile*). On the left, the first author of the study is followed by the reference number in parentheses. The size of the black box corresponding to each study is proportional to the sample size; the horizontal line shows the corresponding 95% confidence interval of the odds ratio. The combined estimate is based on a random-effects model shown by the diamond. The solid vertical line represents the null result: an odds ratio of 1.

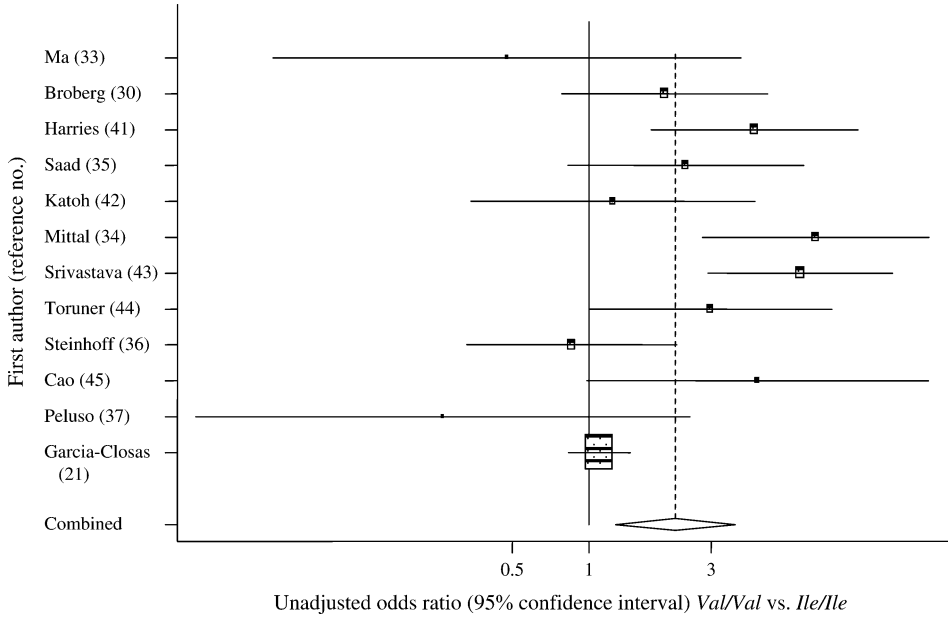

FIGURE 2. Forest plot of unadjusted odds ratios and corresponding 95% confidence intervals for *glutathione* S-*transferase P1 Val 105Val (GSTP1 Val/Val)* versus *glutathione* S-*transferase P1 Ile 105Ile (GSTP1 Ile/Ile).* On the left, the first author of the study is followed by the reference number in parentheses. The size of the black box corresponding to each study is proportional to the sample size; the horizontal line shows the corresponding 95% confidence interval of the odds ratio. The combined estimate is based on a random-effects model shown by the diamond. The solid vertical line represents the null result: an odds ratio of 1.

TABLE 3. Results of the case-only analysis of bladder cancer risk associated with *GSTP1 polymorphisms and interaction with smoking**

No. of cases included†	No. of cases included	OR*	95% CI*	*p* value	*Q*‡	*p* value‡
9	1,111	0.99	0.68, 1.47	0.99	10.89	0.21
6	752	0.90	0.53, 1.52§	0.68	7.43	0.19

* *GSTP1*, *gluthatione* S-*transferase P1* genotype; OR, odds ratio; CI, confidence interval.

† Included studies: for unadjusted OR—three studies from the meta-analysis (Cao et al. (45), Toruner et al. (44), Katoh et al. (42)) and six from the pooled data set (Broberg et al. (30), Golka et al. (20), Hung et al. (31), Mittal et al. (34), Saad et al. (35), and Peluso et al. (37)); for adjusted OR—six studies from the pooled data set (Broberg et al., Golka et al., Hung et al., Mittal et al., Saad et al., and Peluso et al.).

‡ χ^2 test for heterogeneity.

§ Adjusted for age and sex.

TABLE 4. Results of conditional logistic regression of *GSTP1 *Ile/Val and Val/Val* vs. *Ile/Ile,* stratified to *GSTM1* and *GSTT1* genotype†**

	OR*	95% CI*	*p* value	OR‡	95% CI	*p* value
GSTM1						
Null status	0.82	0.65, 1.03	0.08	0.89	0.66, 1.20	0.83
Present	1.00	0.78, 1.27	0.99	0.95	0.69, 1.32	0.94
GSTT1						
Null status	0.83	0.58, 1.20	0.33	0.89	0.55, 1.45	0.83
Present	0.91	0.76, 1.10	0.33	0.99	0.78, 1.27	0.58

* *GSTP1*, *gluthatione* S-*transferase P1* genotype; OR, odds ratio; CI, confidence interval.

‡ Adjusted for age, sex, and smoking status.

† Included studies: for unadjusted OR—three studies from the meta-analysis (Srivastava et al. (43), Toruner et al. (44), and Steinhoff et al. (36)) and seven from the pooled data set (Broberg et al. (30), Golka et al. (20), Hung et al. (31), Ma et al. (32), Mittal et al. (34), Saad et al. (35), and Peluso et al. (37): 1,221 cases and 1,982 controls; for adjusted OR—seven studies from the pooled data set (Broberg et al., Golka et al., Hung et al., Ma et al. (33), Mittal et al., Saad et al., and Peluso et al.): 866 cases and 1,366 controls.

influence (publication year: $p = 0.81$, ethnicity: $p < 0.001$, region: $p = 0.75$, and method of selecting controls: $p = 0.62$). The adjusted summary odds ratio for *GSTP1 Val/Val* compared with *GSTP1 Ile/Ile* was 2.95 (95 percent CI: 1.46, 6.18; $p = 0.004$) ($Q = 33.57$; $p < 0.001$) for eight studies. Meta-regression analyses examining the influences of four covariates found that region and method of selecting the controls had a significant influence (publication year: $p = 0.72$, ethnicity: $p = 0.12$, region: $p = 0.002$, and method of selecting controls: $p < 0.001$).

Pooled analysis

The frequencies of genotypes varied in the controls: *GSTP1 Val/Val* was found in 8 percent of European descendents, 7 percent of Africans, and 3 percent of Asians. The frequency of *GSTP1 Ile/Val* was 40 percent in European descendents, 39 percent in Africans, and 30 percent in Asians. The departure from Hardy-Weinberg equilibrium among all the controls combined was tested and was found not to be statistically significant ($p = 0.79$).

The summary crude odds ratio generated by all eight studies suggested a weak association between *GSTP1 Ile/Val* (OR = 1.14, 95 percent CI: 0.75, 1.75; $p = 0.53$) and bladder cancer, and between *GSTP1 Val/Val* and bladder cancer (OR = 1.68, 95 percent CI: 0.82, 3.45; $p = 0.16$), but the estimates lacked homogeneity ($p < 0.001$ and $p < 0.003$, respectively). The summary odds ratios adjusted for age, sex, and smoking status (never/former/current) for *GSTP1 Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile* were 1.14 (95 percent CI: 0.71, 1.83; $p = 0.59$) and 1.78 (95 percent CI: 0.90, 3.50; $p = 0.09$), respectively. Heterogeneity was suggested ($p < 0.001$ and $p = 0.04$, respectively).

The multiplicative-adjusted odds ratio in a case-only analysis was not significant (OR = 0.90, 95 percent CI: 0.53, 1.52; $p = 0.68$), indicating no evidence of interaction between *GSTP1* genotype and smoking status. The Q statistic showed no significant heterogeneity across the studies ($p = 0.19$) (table 3).

Table 4 shows the results of conditional logistic regression of the *GSTP1 Ile 105Val* genotype, stratified on the *GSTM1* (null vs. present) and *GSTT1* genotype (null vs. present) to investigate the interaction between the different genetic polymorphisms. No interaction between any of the genetic polymorphisms was suggested.

DISCUSSION

This meta-analysis included 16 epidemiologic studies from diverse populations. It found that the *GSTP1 Ile 105Val* polymorphisms appear to be associated with a modest increase in the risk of bladder cancer. The pooled analysis produced very similar results. Although the summary odds ratios were not large, polymorphisms not strongly associated with bladder cancer risk should be considered a potentially important public health issue because of their high population prevalence. Publication bias, which can occur when studies with null or unexpected results are not published, was evident.

Publication may bias the results away from the null. To the best of our knowledge, all available epidemiologic studies of *GSTP1 Ile 105Val* associated with bladder cancer risk published prior to April 2006 were included in this meta-analysis. Furthermore, we sent all the principal investigators of the selected articles and all the participants in the International Bladder Consortium a list of the papers that met our inclusion criteria for the meta-analysis, asking them whether they were aware of any more relevant published or unpublished data. It is common to find that meta-analyses results change in a consistent direction over time, suggesting that early studies provide exaggerated estimates of effect or that initial studies stimulate studies that may be substantially

different in design or quality from the initial studies (53). Hence, it may be that the results are not robust because of the limited number of studies and that future replication of the analyses will generate decreased summary odds ratios.

Because of potential heterogeneity in populations, designs, and analyses, we assumed that the true effects being estimated would vary between the studies in addition to the usual sampling variation on the estimates (within studies). To account for both sources of variation, we used random-effects meta-regression analysis to combine the results from the primary studies. Meta-regression analysis identified region and ethnicity as potential sources of heterogeneity. Results from subgroup analyses suggested that the summary odds ratios were different for Europe, the United States, and Asia. Generally, studies conducted in Asia ascribed a higher risk of developing bladder cancer when *GSTP1 Ile/Val* and *Val/Val* versus genotype *Ile/Ile* were compared. Interestingly, the two influential studies identified were conducted in India (34, 43), whereas the Chinese studies did not have a significant influence on the unadjusted summary odds ratio for *GSTP1 Ile/Val* and *Val/Val* compared with *GSTP1 Ile/Ile* (32, 33). When the analysis was limited to European descendents, the summary odds ratio decreased.

To clarify an association between genotype and cancer risk, sample size is considered a crucial factor. However, restricting our analyses to studies including at least 100 cases and 100 controls did not alter the results substantially. Population controls are considered more representative of the general population (assuming lack of participation bias). Again, however, restricting the analysis to controls from the general population did not influence the results.

Pooled analysis of individual data is preferable to meta-analysis from published data, although some heterogeneity remains, because possible sources of bias can be eliminated (54). By pooling the original data, we were able to adjust the different studies for the confounding factors appropriate to each of them. A strength of the GSEC database is the uniform coding of all data following a standard protocol. Furthermore, we were able to examine gene-smoking and gene-gene interactions. However, some limitations need to be considered. Inclusion bias may have affected our results. Another limitation may be the presence of misclassification in the definition of smoking status and in the laboratory genotyping results (due to different polymerase chain reaction protocols). Because of limited information on exposure to environmental smoking, we were not able to exclude exposed subjects from our ''never''-smokers category. ''Never'' smokers may also have included those with occupational exposures to polycyclic aromatic hydrocarbons and aromatic amines, which may had led to additional confounding.

We found no evidence of interaction between smoking status and *GSTP1 Ile 105Val* genotype, although it is biologically plausible because *GSTP1 Ile 105Val* is involved in the metabolization of various cigarette smoke carcinogens (4). No gene-gene interactions (between the different types of glutathione *S*-transferase) were detected. Analyses of gene-environment or gene-gene interactions raise concerns about adequate statistical power and sample size. Our sample size was sufficient to detect an odds ratio of 3.6 for a multiplicative interaction or 2.7 for an additive interaction with a power of 80 percent (55, 56). Furthermore, it may be that gene-environment or gene-gene interactions differ across different ethnic groups; therefore, pooling across different ethnicities may have decreased the result. Confounding is likely to have occurred, because different ethnic groups smoke different types of cigarettes. It is possible that interactions between genes and other environmental factors (e.g., occupational exposure) may play a role. Assessment of independence between gene and environmental exposure in the general population is conditional for case-only analyses. We did test this assumption on our data in the studies included in the case-only analysis. The control-only odds ratios were close to 1 and were not significant, indicating that the different genotypes do not modify smoking habits.

In conclusion, the *GSTP1* polymorphisms *Ile/Val* and *Val/Val* compared with *Ile/Ile* seem to be associated with a modest increase in the risk of bladder cancer. The associations appeared to be the greatest for Asians. However, there was no evidence of a multiplicative interaction with smoking. Our results must be replicated by conducting follow-up studies of the GSEC database, when more data have been accumulated. Future analyses should address the joint impact of *Ala114Val* genotype or other genetic factors.

LABORATORY TESTS

Nearly all studies included in the present analysis used genomic DNA extracted from blood. Three of them extracted DNA from buccal cells (21, 30, 35). All studies used polymerase chain reaction for genotyping.

POPULATION TESTING AND OTHER PUBLIC HEALTH APPLICATIONS

To date, there is insufficient evidence to identify individuals who have a high risk of cancer because of their increased genetic susceptibility in response to a carcinogenic agent. More evidence is needed from epidemiologic studies to assess the association between *GSTP1 Ile 105Val* and bladder cancer risk to support any public health recommendation. Because tobacco remains the best documented risk factor regarding bladder cancer, the major public health effort should be directed toward prevention and cessation of smoking.

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